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PHYTOCHEMICAL EVALUATION AND BIOLOGICAL ACTIVITY OF AN EPIPHYTIC ORCHID *ERIA LASIOPETALA* (WILLD.) ORMEROD

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Abstract

The current study was undertaken to evaluate the phytochemicals (qualitative and quantitative) and antioxidant activity of leaf, bulb, and root extracts of *Eria lasiopetala*. The qualitative analysis was determined by standard methods and the results confirmed the presence of terpenoids, coumarins, quinines, flavonoids, phenols, and saponins in all the three extracts of this orchid species. Quantitative estimation of total phenol, flavonoid, and tannin contents were ascertained by using standard methods. The maximum amount of phenol, flavonoid, and tannin contents were found in the leaf methanolic extracts as 54.81±7.7 mg $GAEg^{-1}$, 108±1.70 mg QEg^{-1} and 80±5.49 mg TAg^{-1} respectively. DPPH (1,1-diphenyl-2-picryl hydrazyl) radical scavenging assay was used for determining the antioxidant activity of both methanolic crude extracts and four fractions [DCM (Dichloromethane), n-Hexane, Methanol, and Butanol] of leaf and root. The percentage of scavenging of DPPH free radical was found to be concentration dependent. Amongst all the samples, the most significant antioxidant activity was found in Butanol-1 and DCM extracts of bulb (IC_{50} value of 19.22 μgml^{-1} and 23.68 μgml^{-1} respectively). However, the present investigation showed that the *Eria lasiopetala* is a reservoir of the phytochemicals and it can be utilized for the development of phyto-therapeutics.

Introduction

ORCHIDACEAE IS a highly evolved and widely distributed monocotyledonous family with a large number of terrestrial, epiphytic, and saprophytic species. It comprises 28,484 species grouped into 880 genera (Govaerts *et al.,* 2017). They are aesthetically and medicinally important and also regarded as ecological indicators (Chowdhery, 1998; Joshi *et al*., 2009, Kumari and Pathak, 2020). Bangladesh is also rich in orchids with 179 species under 70 genera (Huda, 2008). Eighty five orchid species belonging to 39 genera have exhibited various medicinal properties (Kaushik, 2013; Ghanaksha and Kaushik, 1999; Vaidya *et al.,* 2000). The genus *Eria* Lindl. is one of the larger aggregations of orchids into one genus in Orchidaceae. It is estimated that there are about 500 species of *Eria* in the world (Summerhayes and Hunt, 1973). The stem of the *Eria spicata* can be pounded to make paste with water and applied externally on the forehead to get relieve from headache. The paste can also be taken orally to cure mild stomach ailment (Kumar, 2002). The leaf and root parts of the plant can be boiled and used in the treatment of bone ache. In his two chapters *Orchids in Ancient Indian Literature* and *Medicinal Value of the Orchids* in the monograph, Kaushik (1983) mentioned the formulation of orchids in some Ayurvedic medicines. Phytochemicals derived from orchids are considered as an antioxidant agents which are capable of slowing down or preventing the antioxidant of other molecules such as free radicals or reactive oxygen species (ROS). Antioxidants are widely used as ingredients in dietary supplements and are exploited to maintain health and prevent oxidative stress-mediated diseases such as cancer, atherosclerosis, diabetes, inflammation and ageing, malaria, rheumatoid arthritis, neuro-degenerative disorders (Balkrishna *et al.,* 2020, Devi *et al.,* 2018; Jhansi *et al.,* 2019; Joseph *et al.,* 2018; Kumar *et al.,* 2019; Kumar *et al.,* 2018; Prakash and Pathak, 2019; Vasconcelos *et al*., 2007). Many antioxidants have been isolated from different plant materials and analysed for effective, non-toxic natural compound with antioxidant (Gupta and Sharma, 2006). Isoamoenylin, a dihydrostilbene isolated from roots of *Dendrobium amoenum* var. *denneanum,* showed moderate antioxidative and weak antibacterial activities (Venkateswarlu *et al.,* 2002*).* Some species of Santalaceae (*Osyris quadripartite*, *Quinchamalium chilense,* and *Viscum album*) have been reported as potential free radical scavengers which are correlated with their total phenol content (Vicas *et al*., 2012). Hence, the present investigation was undertaken to evaluate the presence of different phytochemicals and to quantify some of these in the leaf, bulb, and root along with determination of antioxidant activity in different parts of *Eria lasiopetala*.

Material and Methods

Plant materials were collected from Sylhet of Bangladesh. The collected plants were carefully examined and consulted with literature and existing herbarium specimen. A voucher specimen (HCU 135) has been deposited for future reference in the Chittagong

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University herbarium. The plant samples were thoroughly washed with water, cut into small pieces, and then dried at 65°C for 48 hrs. Leaf, bulb, and root of the species were separated for further analysis. Each part was then pulverized into coarse powder mechanically and was stored in air tight container. Approximately 50 g of plant sample from each part was extracted with Methanol separately. After filtering using Whatman no. 1 filter paper and repeating the process, the extract was rotavaporated for drying and kept as crude.

Phytochemical investigations were performed for preliminary screening of the active compounds in the fresh sample, methanolic crude extracts and powdered specimens of leaf, root, and bulb. The five alkaloid detecting reagents used were *i.e.* Mayer's, Hager's, Wagner's, Dragendroff's, and Tannic acid reagents which were prepared by Cromwell's method (Cromwell, 1955). Qualitative test for phlobatannin, flavonoid, saponins, tannin, terpenoids, steroids, glycosides, protein, quinine, and phenol were performed following standard methods (Edeoga *et al.,* 2005; Harbrone, 1973).

Total content of phenol was estimated by Folin-Ciocalteu assay (Harborne, 1998). The absorbance was observed at 760 nm on spectrophotometer (Shimadzu, Japan) and Gallic acid solution was used as standard. The content of phenol in extract was expressed in terms of Gallic Acid Equivalent (mg of GAE g^{-1} of extract).

The quantity of tannin was determined by Folin-Ciocalteu method (Naima *et al*., 2012). Absorbance was measured at 700 nm with an UV/Visible spectrophotometer (Shimadzu, Japan) and Tannic acid was used as standard. The total Tannin content was expressed in terms of mg of Tannic acid equivalents g^{-1} of dried sample.

Total flavonoid was determined using the method of Djeridane *et al.* (2006). Absorbance was measured at 430 nm versus blank using Spectrophotometer (Shimadzu, Japan) and standard was Quercetin. The total flavonoid content was expressed in mg QEg^{-1} of the dried plant extracts. Different concentrations of standard solutions and triplicate samples were prepared for all studied experiments.

Antioxidant activity was determined by free radical scavenging assay of DPPH (Brand-Willams *et al*., 1995). The absorbance of DPPH solution (control solution "A") was measured at 517 nm using UV-visible Spectrophotometer (Shimadzu, Japan) and standard was Ascorbic acid.

The scavenging activity against DPPH was calculated using the following equation:

$$\text{Scavenging activity (\%)} = \left(\frac{A-B}{A}\right)X100$$

A, Absorbance of control (DPPH solution without the sample); B, Absorbance of DPPH solution in the presence of the sample (extract/Ascorbic acid).

In antioxidant assay, the per cent scavenging activity was plotted against log concentration and the IC_{50} (inhibition concentration; 50 $\mu g ml^{-1}$) value was determined by linear regression analysis. The experiments were performed in triplicates. Data were analyzed using one-way Analysis of Variance (ANOVA) followed by Dunnett's test for multiple comparisons. Experimental results were examined further for Pearson correlation coefficient and significance tested using t-test ($p<0.01$, $p<0.05$). All the values are presented as mean with ± Standard error of mean (SEM) of three replicates.

Results and Discussion

Qualitative Phytochemical Analysis

The therapeutic properties of medicinal plants are due to the presence of secondary metabolites (Sangwan *et al*., 2004). *Eria lasiopetala* is a medicinal epiphytic orchid used for the treatment of many human ailments. In the present investigation, 11 phytochemicals such as alkaloids, flavonoids, phenols, tannins, glycosides, saponins,

Table 1. Qualitative tests for other phytochemicals of *E. lasiopetala*.

Plant parts used	Phytochemicals (% of colouration)										
	Alk.	Tan.	Ter.	Str.	Cou.	Qui.	Phe.	Flav.	Phl.	Sap.	Gly.
Leaf	12+	+++	++	_	++	++	+	+++	_	+	_
Stem	9+	++	+++	++	+++	+++	++	+	_	+++	_
Root	10+	_	+++	+++	+++	+++	+++	+	++	+++	++

Alk., Alkaloids; Tan., Tannin; Ter., Terpenoids; Str., Steroids; Cou., Coumarin; Qui., Quinine, Phe., Phenol; Flv., Flavonoid; Phl., Phlobatanin; Sap., Saponin; and Gly., Glycosides.

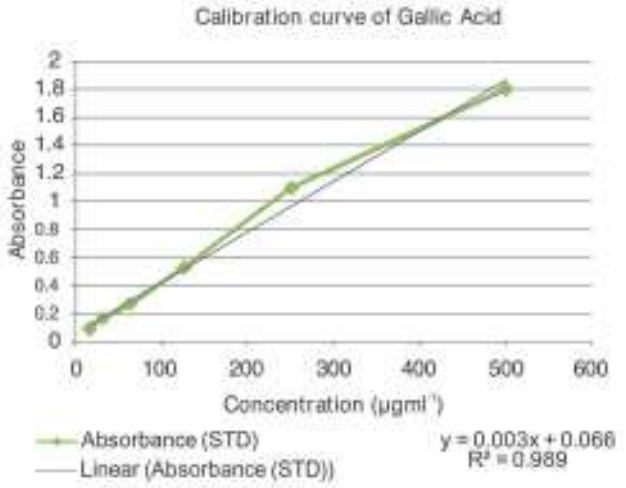

Fig. 1. Calibration curve of Gallic acid for total phenol content determination.

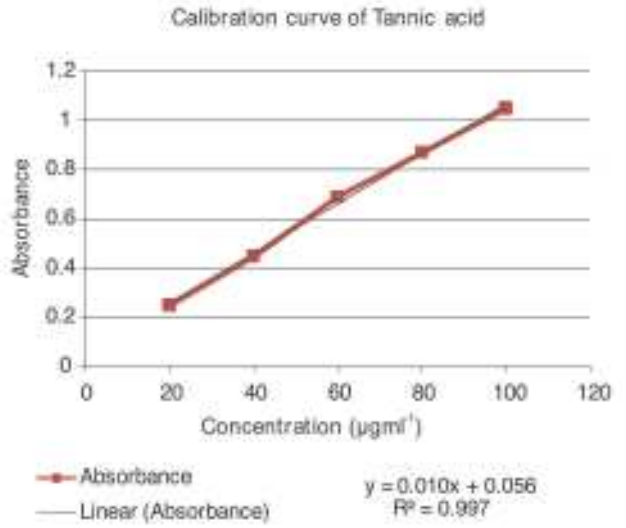

Fig. 3. Calibration curve of tannic acid for total tannin content determination.

quinones, coumarins, terpenoids, and phlobatanin were present in different parts of *E. lasiopetala* (Table 1; Figs. 1-4). The present results are in accordance with the observations of Singh *et al*. (2012) who reported the presence of alkaloids, flavonoids, terpenoids, and stilbenoids, in some orchids.

Antioxidant Activity

Free radicals, which are produced by the chemical reaction or organic compounds, could damage the body's tissues and cells leading to human ageing and causing a variety of diseases. Therefore, it is very important to find the antioxidants for scavenging these free radicals. DPPH method is one of the universal tools for estimating the antioxidant activities of the different products in a short period. DPPH radical and various table phytochemicals given in nitrogen-centered

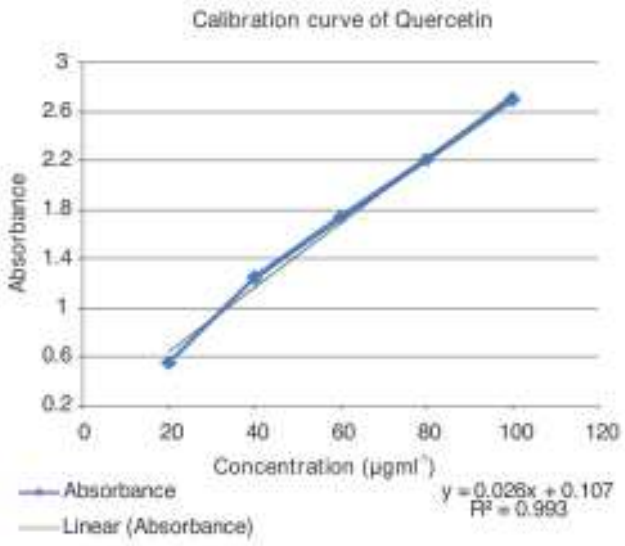

Fig. 2. Calibration curve of Quercetin for total flavonoid determination.

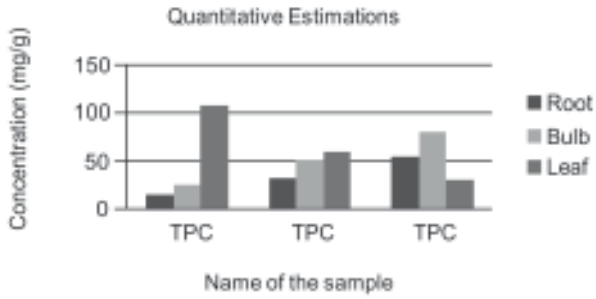

Fig. 4. Total Phenol, tannin, and flavonoid content in leaf, bulb, and root of *Eria lasiopetala*.

radicals, can be used to determine the free radical scavenging ability, which is related to their antioxidant activities.

The antioxidant activity of different methanolic crude extracts and their fraction samples of *Eria lasiopetala* were determined by DPPH free radical scavenging assay. The antioxidant capacity of each extract was measured as the IC_{50} value of the extract, or in other words, the amount required to scavenge 50% DPPH free-radicals. Amongst the five different concentrations (50, 100, 150, 200, and 250 μgml^{-1}) used in the present study, Ascorbic acid (standard) showed 52.09%, 70.55%, 80.89%, 87.12%, and 95.65% scavenging activity respectively, where highest scavenging activity was 95.65% at 250 μgml^{-1} concentration and lowest scavenging activity was 52.09% at concentration 50 μgml^{-1} (Fig. 6). The IC_{50} value of Ascorbic acid is 17.87 μgml^{-1} (Fig. 8).

The highest mean scavenging activity of leaf was 82.07% at the concentration 250μgml^{-1} and the lowest mean scavenging activity was 44.88% at 50

µgml^{-1}concentration. The IC_{50} value of leaf methanolic crude extract is 96.95 µgml^{-1} (Fig. 5).The highest amount of scavenging activity of stem was 82.27% at the concentration 250 µgml^{-1} and the lowest mean scavenging activity was 53.75% at 50 µgml^{-1} concentration. The IC_{50} value of bulb methanolic crude extract is 45.50 µgml^{-1} (Fig. 6). The highest mean scavenging activity was 74.16% at 250 µgml^{-1} concentration and the lowest mean scavenging activity was 49.39% at 50 µgml^{-1} concentration. The IC_{50} value of methanolic crude extract of root is 35.97 µgml^{-1} (Fig. 7).

Amongst all the methanolic crude extracts of *Eria lasiopetala,* the highest antioxidant potential has been found in root sample. The IC_{50} values against DPPH free radical of Methanolic crude extracts were in the following order: Root>Bulb>Leaf; and the IC_{50} values were found as 35.97, 45.50, and 96.95 µgml^{-1} which could be comparable with the Ascorbic acid with an IC_{50} value of

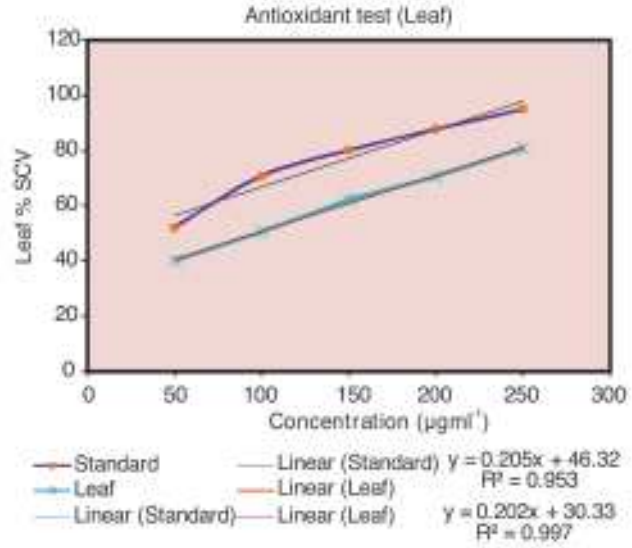

Fig. 5. Relative % scavenging activity (SCV) of Ascorbic acid (As. A-standard) and methanolic crude extract of leaf for antioxidant assay.

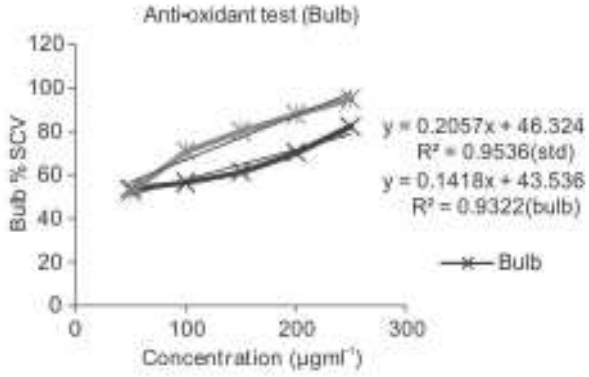

Fig. 6. Relative % scavenging activity (SCV) of Ascorbic acid (As. A-standard) and methanolic crude extract of bulb for antioxidant assay.

18.55 µgml^{-1} (Fig. 8).

The scavenging effects of the leaves Butanol fraction samples IC_{50} values against DPPH free radical were in the following order: Butanol > Methanol> DCM > n-Hexane; the IC_{50} values of the leaves fraction samples were found to be 49.82 > 50.13 > 92.27 >274.07 µgml^{-1} respectively, which could be comparable with the Ascorbic acid with an IC_{50} value of 17.87 µgml^{-1}.

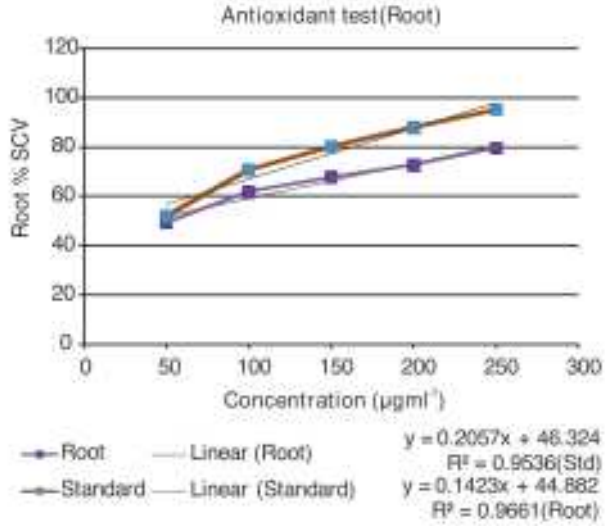

Fig. 7. Relative % scavenging activity (SCV) of Ascorbic acid (As. A-standard) and methanolic crude extract of root for antioxidant assay.

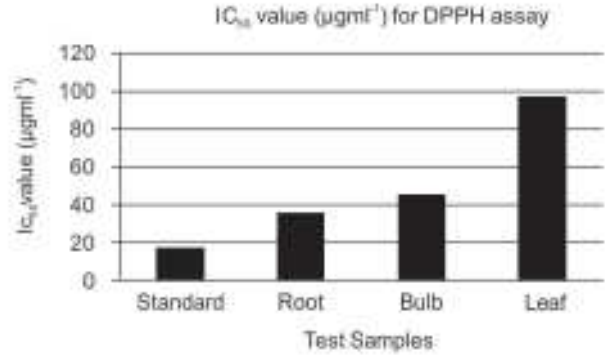

Fig. 8. Comparison of IC_{50} values of the extracts of leaf, bulb, and root with standard Ascorbic acid for antioxidant test.

In case of four fraction samples of stem, the DPPH free radical scavenging activity of Butanol fraction showed highest antioxidant activity with an IC_{50} value of 19.22 µgml^{-1}. Besides, DCM fraction of bulb sample showed significant antioxidant activity with an IC_{50} value of 23.68 µgml^{-1}. The scavenging effects of the stem fraction sample, IC_{50} values against DPPH free radical were in the following order: Butanol>DCM>Methanol>n-Hexane; values were found to be as 19.22, 23.68, 36.78, and 42.8 µgml^{-1} respectively, which could be comparable with the

Ascorbic acid with an IC_{50} value of 17.87 μgml^{-1}. Amongst all fraction extracts of bulb, n-Hexane fraction extracts showed very less antioxidant potential. For the scavenging effects of the root sample, IC_{50} value of the DCM fraction was 54.99 $\mu gml^{-1}$and the against DPPH free radical were in the following order: DCM> n-Hexane>Butanol>Methanol; values were found to be as 54.99>67.75>68.32>116.35 μgml^{-1} respectively.

The present results agree with the report of Chand *et al.* (2016); the authors worked on 13 extracts of 9 wild orchids of Nepal to assess the antioxidant activity and amongst all the orchid species, leaves of *V. cristata* had the highest DPPH radical scavenging activity having the IC_{50} as 79.69 μgml^{-1}. On the other hand, leaves of *Gastrochilus acutifolius* containing IC_{50} value 341.79 μgml^{-1} possessed the lowest DPPH scavenging activity. Williams and Suja (2016) worked on antioxidant potential of a wild epiphytic orchid *Acampe praemorsa*; DPPH radical scavenging activity varied from the minimum inhibition of 60.37±0.011% (25 µl) to the maximum inhibition of 69.74±0.010% (100 µl). Aqueous extract varied from the minimum inhibition of 56.00±0.005% (25 µl) to the maximum inhibition of 58.83±0.011% (100 µl). Ethanol extract varied from the minimum inhibition of 51.01±0.015% (25 µl) to the maximum inhibition of 54.93±0.010% (100 µl). Paudel *et al.* (2018) reported that *Dendrobium moniliforme* contains potential antioxidant activity. He found that the *D. moniliforme* Hexane extract (DMH) showed the highest percentage of DPPH radical scavenging activity (94.48%), followed closely by *D. moniliforme* ethanol extract (DME: 94.45%), *D. moniliforme* acetone extract (DMA: 93.71%), and *D. moniliforme* chloroform extract (DMC: 94.35%) at 800 μgml^{-1} concentration. The antioxidant capacities of DMC, DMA, DMH, and DME measured in IC_{50} values, were much lower (42.39 μgml^{-1}, 49.56 μgml^{-1}, 52.68 μgml^{-1}, and 58.77 μgml^{-1} respectively).

Conclusion

Present phytochemical investigation exhibited the presence of phenols and flavonoids in methanolic extracts of leaves, bulb, and roots of *Eria lasiopetala*. The abundance of flavonoids and phenols in *E. lasiopetala* would be responsible for its antioxidant activity.

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